THE VASO-DILATOR ACTION OF HISTAMINE, AND ITS PHYSIOLOGICAL SIGNIFICANCE.

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THE histamine paradox, which has been the subject of much discussion, may be stated thus briefly. In the cat, which has been the usual subject for experiment on its action, histamine regularly produces, under anæsthesia, a powerful depressor effect, having all the characteristics of a general vaso-dilatation; in perfused organs from the same species histamine has been found habitually to produce, not vaso-dilatation, but vaso-constriction in its place. Explanations of widely different kinds have been offered for this paradox. Dale and Richards(1) satisfied themselves that the effect seen in the anæsthetised cat was a true, peripheral vaso-dilatation, independent of the nervous system. They explained the paradox by the incidence of the effect on the capillaries rather than the arteries, and showed that, under appropriate conditions (presence of blood corpuscles and adrenaline), the vaso-dilator effect could be demonstrated even on the artificially perfused organs of the cat. Other observers have attempted to explain it by supposing that the depressor effect seen *in vivo* was not due to vaso-dilatation. Thus Mautner and Pick(2) attributed it to constriction of hepatic venules. McDowall(3), while admitting capillary dilatation as a factor in the effect, has suggested that constriction of pulmonary arterioles is the earliest and principal agent in the depressor action. Inchley(4) has attributed the effect to constriction of the venules.

In the present paper we present the results of observations undertaken primarily to complete those of Dale and Richards. It was important to know whether the action of adrenaline, mentioned above, was a specific one, or whether other vaso-constrictor agents could replace it, in creating a condition favourable to the vaso-dilator effect of histamine in artificial perfusion. The observations have been extended to other species, and some further experiments have been made on the effects seen with natural circulation.

PART I. EFFECTS OBSERVED WITH ARTIFICIAL PERFUSION.

METHODS.

We constructed a perfusion scheme, having several points of superiority to the somewhat makeshift apparatus which Dale and Richards had at their disposal. It consisted essentially of the following parts, the arrangement of which can be seen from the diagram:

(1) The arterial reservoir, *R*, to which the blood was returned after oxygenation, and in which an oxygen pressure was maintained, which could be raised or lowered to any desired level by the adjustable mercury trap, *T*.

(2) A glass spiral, *S*, leading from *R* through a vessel of water kept at from 41° to 45° C., and serving to warm the blood on its way to the arterial cannula, *AC*.

(3) An electro-magnetic hammer, *H*, by which the thin-walled rubber tube leading from *S* to *AC* was rhythmically compressed, *H* being actuated by a rotatory key, so that the pressure was rendered pulsatile, with a rhythm of about 100 per minute.

(4) An outflow recorder of Condon's type (5), *OR*, into which the blood from the vein cannula, *VC*, was led, and which discharged into a large funnel, *F*, from which the blood passed to the oxygenating chamber, *OC*.

(5) The oxygenating chamber, *OC*, which in the form finally adopted, and used in all the later experiments, was built on the principle of that described by Hooker (6), and later used by Drinker (7) and others. It consisted of a vertically placed glass cylinder, about 45 cm. long and 8 cm. wide. This was narrowed at the lower end to a neck closed by a rubber cork, through which passed the tubes for admitting oxygen and withdrawing the blood. The wide upper orifice was closed by a vulcanite lid, *L*. A short tube through this delivered the venous blood near to the centre of a horizontal vulcanite disc, *D*, kept rotating by a small electric motor. The blood, being thrown centrifugally on to the inner surface of the glass cylinder, flowed down this in a thin film, without frothing, to collect in a pool at the lower end, from which it was withdrawn by an adjustable pump, *P*, which lifted it again to *R*. The oxygen tube opened well above the highest level reached by the collecting blood, and the gas passed freely up through the cylinder, escaping by the openings in the lid. The pump and valves were of glass. A spiral of silver wire lightly smeared with vaseline, attached to the opening of the tube which returned blood to *R*, prevented frothing.

It will be obvious that, with such a system, perfusion is carried out

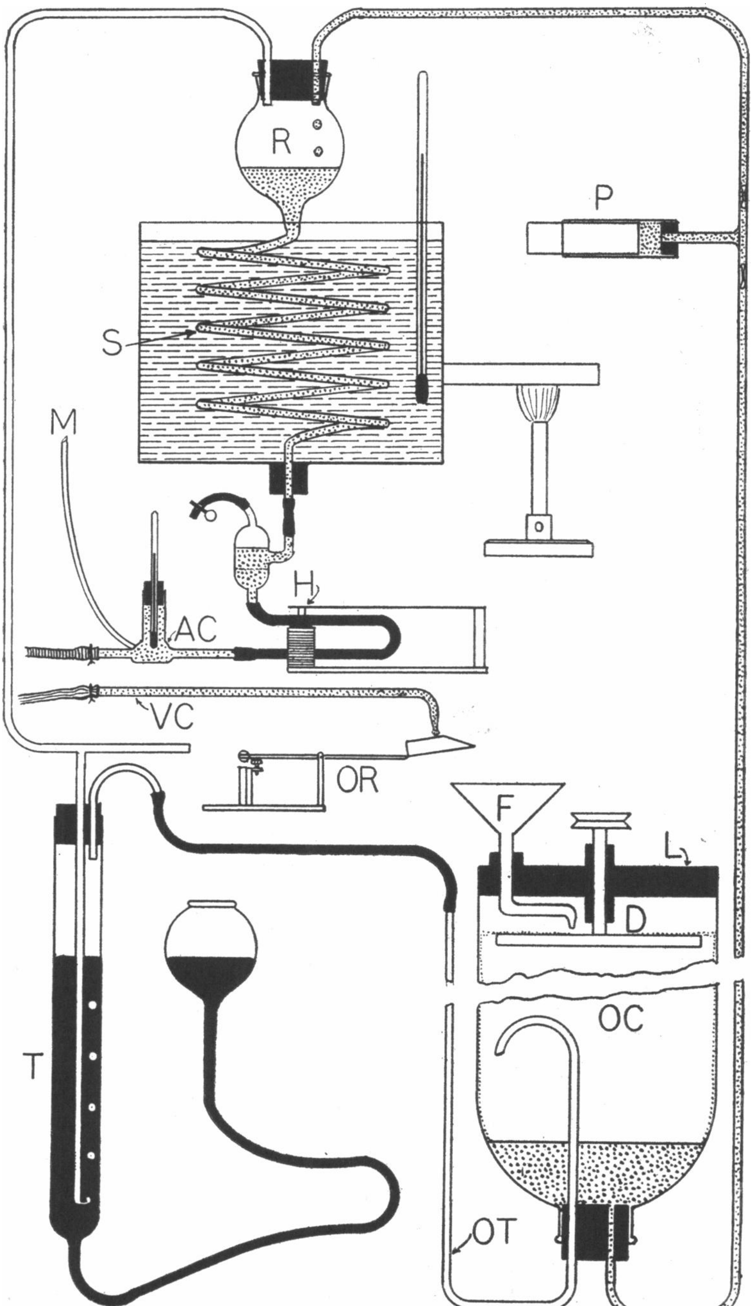

Diagram of Perfusion Scheme.

with a head of pressure, rhythmically cut off by the hammer. The records from the manometer, attached to the arterial cannula, show that the sustained, but pulsatile pressure so produced is very similar to the natural arterial pressure. It has, from our point of view, the advantage that it is but slightly altered by changes in the peripheral resistance, so that the effects of these on the venous outflow and the volume of the perfused organ are practically uncomplicated by secondary changes in the arterial pressure.

The blood used was that of the species, and usually that of the individual, from which the leg for perfusion was obtained. Hirudin being no longer available, a few experiments were made in which coagulation was prevented by heparin or by novirudin (a humic acid preparation), on the supposition that defibrination of the blood might interfere with the demonstration of the effects we desired to study. Experience showed that this supposition was not justified, and we subsequently adopted defibrination as a routine procedure. Under ether anæsthesia the animal was bled through a cannula in the abdominal aorta (cat or monkey) or carotid artery (dog). A fairly complete exsanguination was secured by injecting Ringer's solution into the jugular vein in the later stages, about 50 c.c. being so injected into a large cat and 100 c.c. into a dog. The blood was defibrinated by stirring with feathers and filtered through cotton-wool. About 150 c.c. of blood sufficed to fill the apparatus, and this quantity, slightly diluted by the saline infusion, could easily be obtained from a large cat. When smaller cats were used, two were killed to obtain blood for the perfusion of a limb from one.

When Ringer's solution with gum was used it was prepared by a modification of the method recommended by Bayliss(8). A 20 p.c. solution of gum arabic in water was made, and to this was added phosphoric acid in the proportion of 0·0268 c.c. of $M/10$ acid per c.c. The mixture was then brought to the alkaline side of the neutral point (pH = 7·4) by addition of normal sodium hydrate, and, after standing awhile to aggregate, was filtered. To the clear filtrate was added 0·9 p.c. of NaCl. One part of this strong gum-saline was diluted with two parts of ordinary Locke's solution, so that the mixture contained the ions of Locke's solution in approximately normal proportions, and, in addition, between 6 and 7 p.c. of gum. It was thoroughly saturated with oxygen before introduction into the apparatus.

Cannulæ were tied into the femoral artery and vein without undue delay; but experience showed that great hurry was not needed. The external pudic and deep femoral vessels were tied off, and other branches to the thigh muscles in many instances, the abdominal viscera were removed, and the body wall mass-ligatured with strong twine in three sections. The body was then transected above the mass-ligatures and the spinal canal firmly plugged with plasticine. As perfusion proceeded there was usually some loss of blood into the tissues above the area deliberately perfused, but this was minimised by the precautions mentioned to close anastomotic paths. The cannulæ being connected to the perfusion apparatus, the perfusion was started. The plethysmograph was then pushed on and filled, during observation of the venous outflow, care being taken that the cuff exercised no pressure sufficient to interfere with the freedom of circulation. The plethysmograph used was a glass cylinder with an invaginated cuff of soft rubber, making water-tight contact with the vaselined leg. It was filled with warm water, the temperature of which was maintained by an adjacent carbon-filament lamp. The plethysmograph, when adjusted, was clamped firmly to the table. Air connection was made with a small Brodie's bellows.

Injections were made directly into the arterial cannula, the needle being pushed through the rubber tube leading to it. Usually the drug was dissolved in Ringer's solution, in such concentration that the doses used were contained in from 0·05 to 0·2 c.c. The 1 c.c. syringe, containing this small volume, was allowed to fill with warmed blood under the pressure in the arterial cannula, and the mixture was then returned into the blood-stream. In order to ensure that even this slight dilution did not produce fallacious small accelerations of venous outflow, owing to reduced viscosity of the circulating blood, the effects were repeatedly controlled with solutions made up with a small volume of blood taken from the apparatus, instead of with Ringer's solution. The effects were not altered in any way by this procedure, nor did the similar injection of corresponding volumes of plain Ringer's solution produce any perceptible effect. When perfusions were made with gum-Ringer solution, the drugs were dissolved for injection in a sample of this solution.

The lines of record on the smoked paper showed in each case, (1) the leg-volume, (2) the perfusion pressure, (3) the rate of venous outflow, (4) the time in intervals of 10 seconds.

Results.

(1) *Perfusion of the cat's leg.*

(*a*) *Perfusion with plain blood.* Dale and Richards had found that neither plain blood, nor gum-Locke solution containing adrenaline, would maintain a tone in the peripheral vessels which histamine would relax. Only when red corpuscles and adrenaline were present together in the perfusing fluid did they observe the vaso-dilator effect of histamine. Our original object was to test the possibility of replacing adrenaline in this combination by other vaso-constrictor agents. The investigation has been given a somewhat different form by the fact that, when blood was used, we obtained evidence of a vaso-dilator effect of histamine, before any artificial addition of adrenaline or other vaso-constrictor substance was made, in all but the first experiment of the present series. The results in this first experiment correspond with those of Dale and Richards, histamine producing apparently a simple vaso-constriction during perfusion with plain blood, and a simple vaso-dilatation after adrenaline had been added and the perfusion pressure suitably raised. In every other case the first small injection of histamine (usually 0·005 mgm.), and in many cases several succeeding similar injections, have produced a decided acceleration of the venous outflow, though blood unmodified, save by whipping or adding heparin, was being used for the perfusion.

The plethysmographic changes accompanying this acceleration have been less constant, especially in the earlier experiments on the leg with skin intact. Out of the first 16 experiments of this kind, in only five was the acceleration of outflow accompanied by a significant increase of the leg volume, while in some cases a slight diminution of volume was apparent. An explanation of this discrepancy was suggested by the results of five other experiments, in which we perfused the leg from which the skin had been removed, the foot being amputated at the ankle-joint. All severed vessels were carefully ligatured, and for these experiments the plethysmograph was filled with warm Locke's solution instead of water. In this series a first small dose of histamine produced in every case a definite increase of volume, accompanying the acceleration of venous outflow and subsiding as it disappeared. Dale and Richards had shown that, in the intact animal also, the dilator effect of histamine is exhibited more regularly in the vessels of the muscle than in those of the skin. In recording the volume of the perfused leg it is not practicable to include in the plethysmograph the whole of the perfused portion, and, when the skin is left on, the part in the plethysmograph necessarily includes the foot, with much skin and little muscle, while the part outside consists largely of thigh muscle. Under such conditions, if we suppose that the vessels of the skin pass more readily than those of the muscles into the condition in which histamine can no longer produce its vaso-dilator action, but merely constricts the arteries, it is easy to understand the production, on balance, of an accelerated venous outflow from the whole area perfused, with no expansion, or even a small shrinkage, of the part yielding the volume record.

Our more recent experience, however, has shown that even in the limb with skin intact, perfused with plain blood, it is possible to demonstrate in practically every case the complete vaso-dilator phenomenon—accelerated venous outflow and expansion of volume—in response to a small injection of histamine given at the right period of the perfusion. In our last five experiments, succeeding the series of 16 above mentioned, we have observed it in every case. Figs. 1 *A* and 2 *A* show records

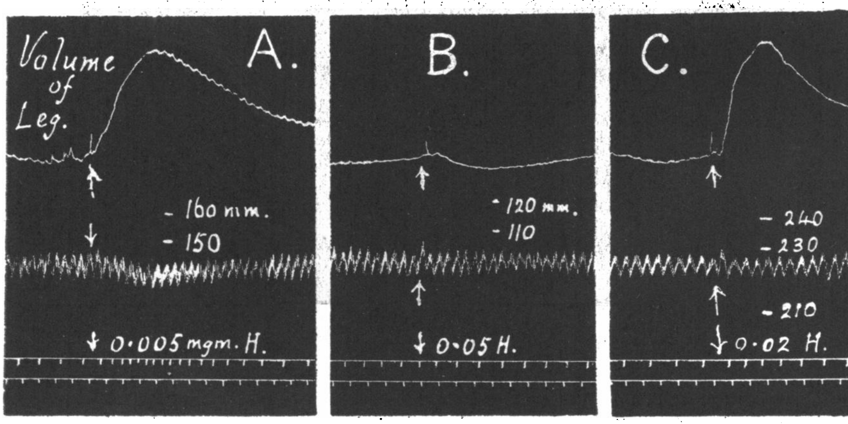

Fig. 1. Perfusion of cat's leg with whipped blood. Effects of histamine, (*A*) during initial vascular tone, (*B*) later, (*C*) again later, after adding 1 in 5 millions adrenaline. In this and other figures *AC* = acetyl-choline, *H* = histamine.

obtained from a leg with the skin intact, and from skinned leg muscles respectively. The effects appear to be those of perfectly normal vaso-dilatation, such as could be expected from the effects of histamine seen in the living animal. Fig. 2 *A* shows, for comparison, the effect of a small dose of acetyl-choline; the histamine effect differs from this in its time-relations, but is comparable in all other obvious respects.

The possibility being thus demonstrated of observing the vaso-dilator action of histamine in an organ perfused with plain blood, it is natural to enquire as to the reason of our present success, and of the uniform failure of earlier workers, including one of us in association with others. We are not confident of being able to give a complete answer. Speed in completing the preparation, and beginning the artificial after cessation of the natural circulation, is certainly not a factor. Dale and

Richards found that transfer from one to the other without even momentary interruption was not effective. We have begun by exsanguinating the animal, and have then made the necessary dissections

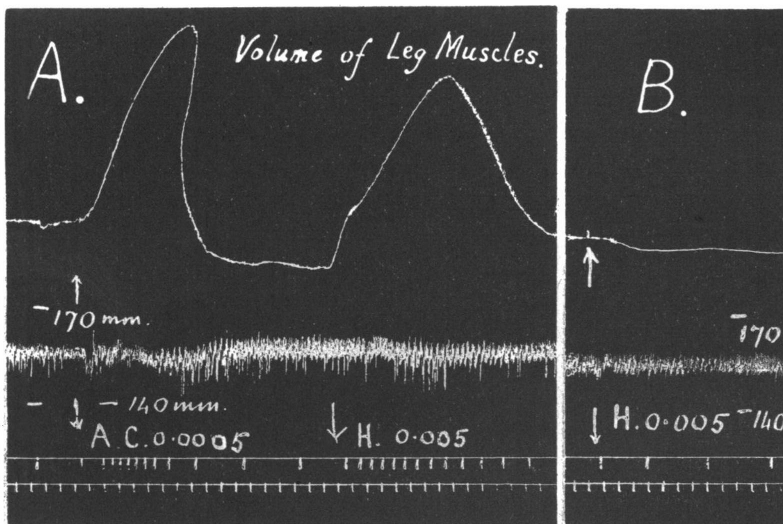

Fig. 2. Perfusion of skinned leg with whipped blood. Effects of acetyl-choline, and of histamine (*A*) early in the perfusion, (*B*) later, after a larger dose of histamine.

and adjustments without hurry, though without undue delay. The tissues have regularly been without circulation for some 15–20 minutes. In our later experiments we have been more careful to tie off such branches in the thigh as could easily be reached, so as to make the part perfused correspond more nearly with that in the plethysmograph, and have probably been more successful in securing a fit of the plethysmograph without pressure. These measures have probably played a part in producing a closer correspondence between the recorded changes in volume and in outflow, but they do not explain the acceleration of outflow regularly observed in practically all the experiments.

So far as we are able to judge, the chief factor in the success of our demonstration has been as follows. Having a perfusion scheme well under control, and working with a minimum of attention, we have been able to begin the record practically with the start of the perfusion and to watch the progress of events. At the outset the vessels of the leg are fully relaxed, but when the perfusion has been in progress for a few minutes signs of a spontaneous recovery of tone appear. The perfusion pressure has to be raised progressively, frequently to 150 mm. of mercury or more, as the resistance increases, in order to maintain an efficient circulation. A plethysmograph record covering this period shows that a rapid shrinkage of the organ accompanies the fall in venous outflow. Presently outflow and volume become practically constant, without further change of perfusion pressure, and this condition may persist with little change for a varying period up to 20 minutes. Sooner or later, even if no injection is made, this spontaneous tone begins to subside and the pressure has to be lowered again to maintain the perfusion within reasonable limits of speed. Fig. 3 illustrates this phenomenon, as seen

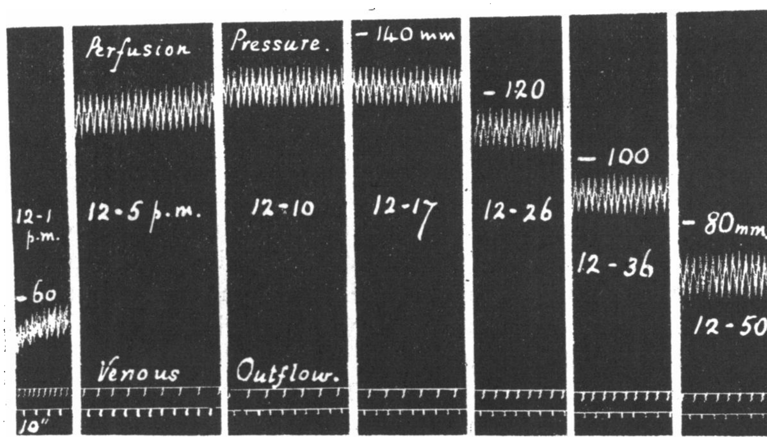

Fig. 3. Acquisition and loss of spontaneous tone by vessels of a cat's legs during the first hour of a perfusion with plain, whipped blood.

in a perfusion of both hind limbs of a cat, made for another purpose. Comparison of the height of perfusion pressure with the rate of outflow at different periods will show the rapid onset and gradual subsidence of the vascular tone. It is during this early period of the perfusion, in which it seems reasonable to regard the tone of the perfused vessels as similar to that which they exhibit in the body even after denervation, that the histamine dilatation can readily be demonstrated. After it has passed, histamine will produce simple vaso-constriction. Further, histamine itself in somewhat larger doses, such as 0·02 mgm., accelerates the disappearance of the spontaneous tone. If histamine, therefore, is given, in more than minimal doses, either too early or too late in the perfusion, the development of this natural tone, and its relaxation by histamine are easily missed altogether; and we think it probable that this circumstance explains the earlier failures to observe it. Figs 1 and 2 *B* are taken from later stages of the same experiments as Figs. 1 and 2 *A*. After the effect shown in Fig. 2 *A* a dose of 0·02 mgm. of histamine was given and produced a prolonged vaso-dilator effect, the vascular tone never being regained. Further injections of histamine produced simple vaso-constriction, as shown in Fig. 2 *B*. When once the tone which histamine relaxes has thus been lost, we have never observed its spontaneous return under further perfusion. Thereafter, other vaso-dilators, such as acetyl-choline, continue to produce their normal effects, while histamine as regularly produces vaso-constriction as long as the preparation remains in condition to demonstrate vascular reactions.

Our problem, therefore, was to compare the effect of different vaso-constrictor substances added to the perfusion fluid, not in producing initially a vascular tone which histamine would relax, since this could occur spontaneously, but in restoring such tone when it had finally disappeared.

(*b*) *Effects of added vaso-constrictor substances. Adrenaline.* Our experience with this substance uniformly confirmed that of Dale and Richards. Adrenaline has never failed to reproduce such a condition in the vessels that histamine would again exhibit its vaso-dilator effect (Fig. 1 *C*), even in larger doses, up to 0·1 mgm. In several experiments adrenaline has succeeded after other vaso-constrictor substances had failed. The concentration necessary has varied with the condition of the preparation. If this is good, as little as 1 part in 20 millions suffices. On occasion a concentration such as 1 in 5 millions has not been adequate, and it has been necessary to raise the strength to 1 in 2 millions, raising the perfusion to 200 mm. of mercury or more, in order to obtain an adequate perfusion rate against the high resistance thus produced. Only under two conditions have we known adrenaline to fail, viz. when sufficient ergotoxine had been given to paralyse its vaso-constrictor effect, and when, as the result of long perfusion, the tissues had become so œdematous that, when vaso-constriction with adrenaline was produced, further perfusion became impossible.

Pituitary (posterior lobe) extract. It was important to examine the effect of pituitary extract, because it contains the only known potent vaso-constrictor principle, other than adrenaline, occurring naturally in the body, and because Krogh and his co-workers (9) had shown that this principle had a tonic action on the cutaneous capillaries of the frog. We made five experiments in which the extract was added to the perfusing blood in quantities corresponding to one part of dry, acetone-extracted posterior lobe to 40,000 of blood. In most cases an obvious vaso-constriction was produced, so that the pressure had to be raised to maintain a good rate of perfusion. In two cases a previous constrictor effect of histamine was converted into the normal, dilator effect. One of these is illustrated in Fig. 4.

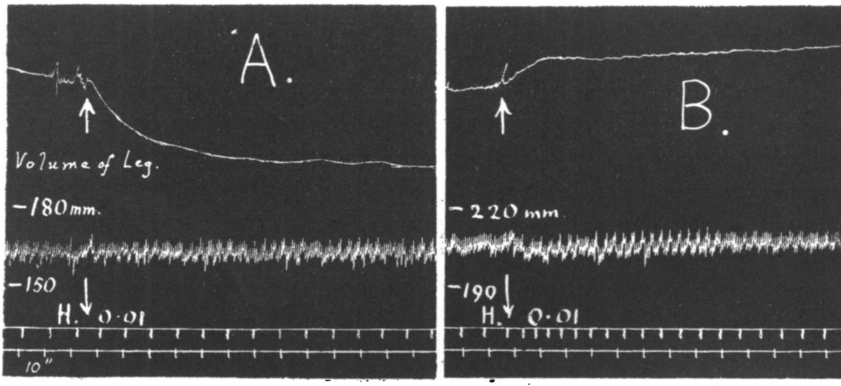

Fig. 4. Restoration of dilator response to histamine by adding pituitary extract to perfusing blood.

There can be no doubt, therefore, that the pituitary principle can restore the lost tone to those vessels on which histamine exercises its dilator action. Its effect in this direction cannot, however, be demonstrated with anything like the same ease and regularity as that of adrenaline. In two of the three other experiments, in which pituitary extract completely failed to effect this restoration, adrenaline was subsequently added, with the usual success.

Barium chloride. This vaso-constrictor substance was tried in one experiment only. It was added to the perfusing blood so as to produce a concentration of 1 part of $BaCl_2$ in 10,000. A great increase of peripheral resistance resulted, showing that the barium was exercising a potent vaso-constrictor action. In spite of this the vaso-constrictor effect of histamine was unchanged. The conclusion seemed to be justified that, under the conditions of artificial perfusion, the vascular tone produced by barium did not involve the vessels which histamine dilates.

Ergotamine. This alkaloid of ergot, shown by Dale and Spiro (10) to be identical in action with the earlier studied ergotoxine, produces in the spinal animal a general, peripheral vaso-constriction of great intensity and persistence. We observed no vaso-constrictor effect following its injection into the arterial cannula leading blood to the perfused organ.

We can offer no adequate explanation for its failure to appear. Whatever the reason, the injection was followed by a slight decrease of the peripheral resistance. The paralytic effect, however, on the vaso-constrictor action of adrenaline was well displayed. If, subsequently to the injection of 2 mgm. of ergotamine, adrenaline was injected into the arterial cannula in doses up to 0·1 mgm., each injection was followed by a prompt acceleration of the venous outflow, accompanied by expansion of the leg volume. On the other hand, the vaso-constrictor effect of histamine was not at all affected by ergotamine, except in the sense that the presence of the latter prevented adrenaline from producing its normal vascular tone, so that it could not restore the histamine dilator effect.

(c) *Perfusion with Locke's solution containing gum-arabic and adrenaline.* When Locke's solution containing gum-arabic alone is perfused there is no spontaneous development of vascular resistance, and histamine produces simple vaso-constriction. Dale and Richards found that even addition of adrenaline to such a mixture failed to evoke the vaso-dilator action of histamine. Here, again, we have been more successful. In two out of three experiments, in which perfusion was begun with the gum-mixture containing 1 part of adrenaline in 10 millions before the effect of histamine was tested, the first few small injections of histamine produced typical and complete dilator effects. Fig. 5 *A* is from one of

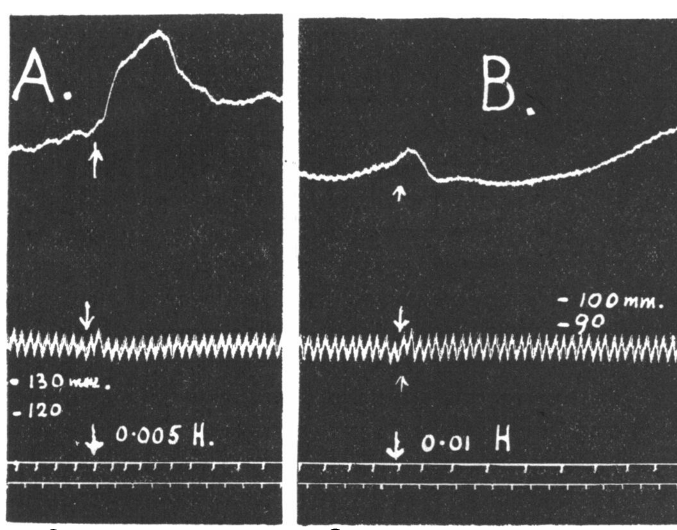

Fig. 5. Cat's leg perfused with gum-Locke solution containing adrenaline 1 in 10 millions. Initial dilator effect of histamine (*A*), disappearing later (*B*).

these. In the third experiment histamine from the first produced a constrictor effect. In the other two experiments the vaso-dilator effect of histamine disappeared after the first few injections, being replaced by constriction (Fig. 5 *B*); and when this had once occurred the vaso-dilator effect could not be restored, even though further adrenaline was added, or the whole of the circulating fluid replaced by a fresh lot, containing adrenaline as before. The vaso-dilator action of acetyl-choline remained unaltered throughout.

(*d*) *Perfusion of isolated arterial branches.* We show later that even separated arterioles of the dog show a trace of dilator response to histamine. It was important, therefore, to repeat the observation of Dale and Richards on those of the cat. Using the preparation of the cat's mesenteric arteriolar branches which they described, we completely confirmed their finding, obtaining, even with the smallest doses of histamine (0·005 mgm.), only a constrictor reaction even when the tone was so raised by addition of adrenaline to the blood, that a pressure of 200 mm. of mercury was needed to procure an adequate rate of perfusion. Fig. 6 *A* shows this effect, and that of a small dose of acetyl-choline for comparison.

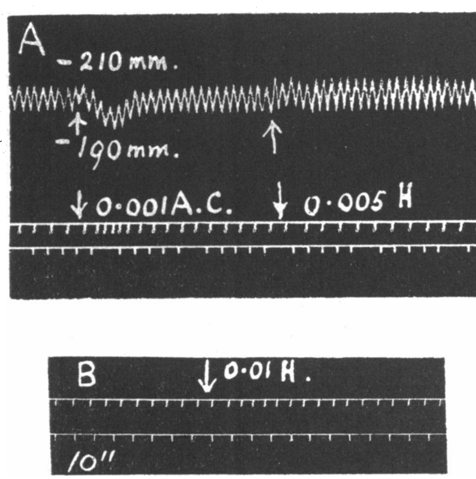

Fig. 6. Effects of histamine on mesenteric arterioles of (*A*) cat, perfused with blood containing added adrenaline, (*B*) dog, perfused with plain blood.

Full discussion of the meaning of these results may be deferred till those obtained in other species have been described. It is sufficient to indicate here that they are by no means opposed to, but rather reinforce the conclusion reached in earlier investigations, that the vaso-dilator effect of histamine in the cat is produced mainly, if not entirely, on the capillaries.

(2) *Perfusion of the dog's blood vessels.*

We have perfused a dog's leg, with the animal's own whipped blood without artificial addition of a vaso-constrictor substance, in 5 experiments. The vaso-dilator effect of histamine was in every case pronounced, and was shown both in the acceleration of the venous outflow and in the increase of volume recorded by the plethysmograph (Fig. 7 *B*). In

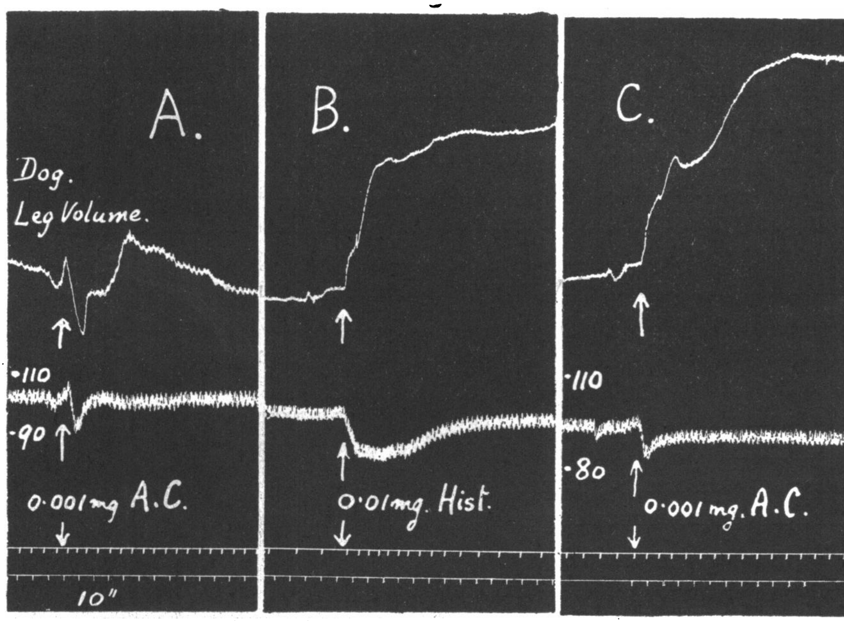

Fig. 7. Dilator effects of acetyl-choline and histamine on vessels of a dog's leg perfused with blood. *C* shows improved effect of acetyl-choline after histamine.

several ways this vaso-dilator action of histamine on the artificially perfused organ of the dog differs from that seen under like conditions in the cat. No special precautions and no choice of a favourable moment appear to be necessary for its observation. It appears to be obtainable at any time, and exhibits no special evanescence. Nevertheless, if records of experiments in which acetyl-choline and histamine are injected alternately are carefully studied, in the light of what is known of the action of both on the cat's vessels, they are found to have features suggesting that the two actions are not identical in localisation. An initial injection of 0·001 mgm. of acetyl-choline was found in several cases to produce a surprisingly small and evanescent effect on outflow and on volume (Fig. 7 *A*). If this was followed by an injection of 0·01 mgm. of histamine, which from analogy with their respective actions on the cat should be roughly equivalent, the effect was found to be much more intense and persistent. If now, when outflow and volume were once more steady, a second dose of 0·001 mgm. of acetyl-choline was given, the result was conspicuously greater than that produced by the first (Fig. 7 *C*). The sequence suggested that the histamine had weakened a resistance peripheral to the point of action of acetyl-choline, enabling the latter now to produce its full effect.

Though such indications suggested that histamine dilated capillaries in the dog, as in the cat, the regularity with which its vaso-dilator effect could be demonstrated, even after prolonged perfusion and repeated dosage, suggested that the arterioles in the dog were also involved in the action. This suggestion was directly confirmed by perfusion of a prepara tion made from the dog's superior mesenteric artery with its fine arterial branches. The conditions were similar to those employed for the corresponding preparation made from the cat. Two such experiments were made, and in each case injection of a small dose of histamine caused a small, but definite acceleration of the outflow from the cut ends of the arterioles (Fig. 6 *B*). It will be seen that, even in the absence of added adrenaline, the small arteries of the dog exhibit a tone which histamine relaxes.

On the other hand, strips cut from larger arteries of the dog, such as the iliac or carotid arteries, and suspended in oxygenated Locke's solutions, always responded to histamine by contraction (Fig. 8).

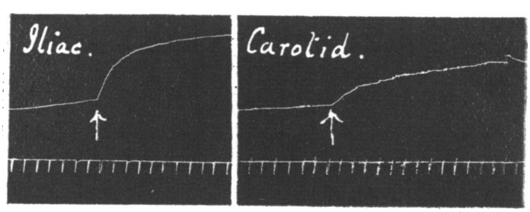

Fig. 8. Isolated strips of large arteries of dog. Constrictor effects of histamine (1 in 200,000).

The difference between the action of histamine on the vessels of the dog and the cat would, therefore, seem to be of this kind, that the change from constrictor to dilator action takes place at different levels in the vascular branching. In the dog it occurs so early that arteries still macroscopically recognisable as such are already involved in the dilatation, whereas in the cat the change occurs at some more peripheral point.

(3) *Perfusion of the monkey's leg.*

We have had the opportunity of carrying out artificial perfusions on the legs of only two monkeys, but the results enable us to state that the effects of histamine on the vessels of this species resemble those in the dog rather than those in the cat. The perfusions were carried out with the plain whipped blood of the monkey from which the leg was obtained, and histamine regularly produced a conspicuous vaso-dilator effect (Fig. 9), which showed no signs of disappearing or changing to vaso-constriction with repeated injections or continued perfusion. The evidence strongly suggests that in the monkey, and therefore probably in man, the dilator effect of histamine is not restricted to the capillaries, but extends to the arterioles, as in the dog.

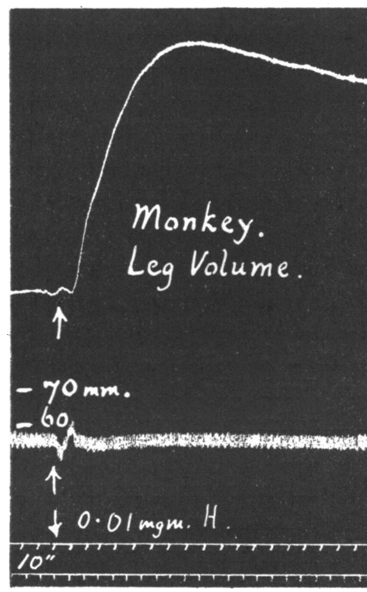

Fig. 9. Effects of histamine on vessels of monkey's leg perfused with blood.

(4) *Discussion.*

The experiments above described have shown that in species, other than the cat, which show a vaso-dilator response to histamine under conditions of natural circulation, this response can be demonstrated under artificial perfusion with the same ease and certainty as that of other vaso-dilator agents. The suggestion that the depressor effect of histamine was due to effects of other types would presumably not have arisen, but for the accident that the cat, rather than the dog or the monkey, was chosen for the early experiments on the nature of its action. Even in the perfused vessels of the cat, under conditions as nearly normal as they can be made, our experiments show that the dilator response to histamine is not really wanting, though very easily lost. When we find that the cat also differs from the dog, in that the finest separable arteries are constricted by histamine in the cat and dilated in the dog, it is natural to associate the two points of difference, and to suppose that the evanescence of the dilator effect in the cat is due to its incidence mainly on the capillary vessels. This is substantially the conclusion reached by Dale and Richards; our evidence only differs from theirs in showing that the dilator response of the cat's capillaries is not unobtainable, as they supposed, if the perfusion fluid does not contain both red corpuscles and added adrenaline, but only peculiarly short-lived.

This conclusion raises the question as to the nature of the agent in the shed blood which, for a time, restores a tone to those vessels in the perfused organ of a cat which histamine relaxes. We have suggested that this tone is comparable to that which the vessels acquire in the body shortly after denervation. It would be natural in the latter case to suggest that the vaso-constrictor hormones, adrenaline and the pituitary pressor principle, are the agents concerned. If that suggestion is accepted, it is equally natural to suppose that traces of these hormones in the shed blood are responsible for the temporary tone which appears in the early stage of a perfusion. Of the two, adrenaline appears, on the evidence before us, to be the one which is likely to be the more important factor. Krogh, indeed, has shown that the capillaries of certain tissues in the frog, though only of some, are highly responsive to the tonic action of the pituitary principle; and several observers, among them Sacks (11), have described a peculiar sensitiveness of the skin capillaries in man to this hormone. On the other hand, our experience with the perfused organ of the cat suggests that adrenaline has, in this species, a unique effectiveness in reviving the tone of the vessels dilating with histamine, when once this has been lost. Pituitary extract will sometimes effect this revival, but only in such degree as to suggest rather a potentiation of the effect of a remnant trace of adrenaline, than a direct and specific action of its own. The evanescence of the naturally acquired tone, and its regular and prompt return when adrenaline is added, accord best with the view that this same, unstable substance is responsible throughout for its appearance.

There are, indeed, difficulties in the way of attributing to adrenaline this chief rôle in the maintenance of tone in the denervated vessels of the living animal, whether arteries or capillaries. Chief among these is the fact, already discussed by Dale and Richards, that adrenaline itself, injected into the circulation in very small doses, produces a vaso-dilator reaction of such vessels, very similar to that produced by a minute dose of histamine. The point can better be discussed later, in connection with experiments on the whole animal, which further illustrate the intimate nature of the antagonism between adrenaline and histamine in their vascular effects. Here we may note, in passing, that we have found it impossible to demonstrate a vaso-dilator effect of adrenaline on the perfused leg of the cat or dog, however high the initial tone of the vessels, however pronounced their vaso-dilatation in response to histamine, and however small the dose of adrenaline injected. The latter, if large enough to produce any perceptible effect, has always produced an uncomplicated vaso-constriction. Only when ergotoxine (ergotamine) had been previously injected could we obtain a vaso-dilator effect of adrenaline on the perfused organ.

Another point calling for comment is the rôle of the red corpuscles in facilitating the demonstration of the dilator effect of histamine on the perfused organ. Dale and Richards had found that red corpuscles must be present and that adrenaline must be added. While our experiments do not strictly confirm this conclusion, showing that either gum solution containing adrenaline or plain blood will often suffice, they do show that the presence of red corpuscles has an effect definitely favourable to the vaso-dilator response to histamine. When they are present, adrenaline can always restore it easily when it has disappeared; with gum solution and adrenaline the effect may be shown early in the perfusion, but, when once it has disappeared, further doses of adrenaline apparently cannot revive it. Dale and Richards attributed the favouring action of red corpuscles to more efficient oxygenation of the tissues; and this may, indeed, be a factor in their influence. It seems probable to us, however, that the explanation is rather to be sought in mechanical considerations, arising from the observations of Krogh, which have since become available. Krogh showed that in a large part of the capillary vessels of the body, and particularly in those of the resting skeletal muscles, a condition of tone normally exists, such that the lumina, even of the small proportion open at all, are so narrow that the elastic red corpuscles must be deformed to pass through them. Under such conditions, even though the total area of the open capillary path may be large in relation to that of the arteries, it appears certain that a material part of the peripheral resistance must be in the capillaries, and that even a slight relaxation of these will produce large effects on the venous outflow and the volume of the organ. Even a moderate, normal capillary tone would apparently suffice to produce these phenomena when corpuscles are present. In their absence, on the other hand, the total area of the capillaries may well be so great that no important part of the peripheral resistance is produced in them, unless their tone is abnormally high, so that few are open at all.

The fact that the small arteries are dilated by histamine in the dog, and probably in the monkey, makes it difficult to obtain evidence from perfusion experiments as to its action on the capillaries in these species. Evidence of other kinds is not wanting, however. Dale and Laidlaw(12) found that histamine in larger doses produced a permanent, shock-like collapse of the circulation, with oligæmia and rise of corpuscular content, in the dog as in the cat. Such an effect has not been demonstrated with any simple arterial dilator, and it is unlikely to be produced by different mechanisms in the two species. More direct evidence is afforded by the observations of Abel and Geiling(13) and Geiling and Kolls(14), who described the vivid general erythema produced in a white-skinned dog, when a small dose of histamine or the similarly acting albumose was given intravenously, and observed and photographed with the microscope, in the latter case, the dilatation of capillaries and venules causing it. The observations of Sollmann and Pilcher(15), and especially the more recent work of Lewis and Grant(16), have shown that histamine directly dilates the skin capillaries in man. In the frog Killian(17) has shown, by direct microscopic observation and photography of the vessels of the tongue, that the dilator action of histamine extends to arterioles and capillaries. In the fowl histamine has a depressor action, superficially quite similar to that which it shows in other species, and this can safely be assumed to be vaso-dilator in type. Among the vertebrates hitherto examined, the rodents (rabbit and guinea-pig) appear to form a conspicuous exception. There is no clear evidence that histamine exerts a vaso-dilator action on any part of the vascular system in these. A constrictor effect on systemic and pulmonary arterioles is easily detected, and the action on the pulmonary vessels may be so severe as to cause acute dilatation of the right chambers of the heart—an effect seen also in the cat as a passing phase in the action of large doses. But the vaso-dilator effect cannot be detected in the rodents, at any rate under conditions which are suited to its demonstration in other species.

So far as its effects on the arterial side of the circulation are concerned, the action of histamine may be pictured, in general terms, as consisting of a constrictor effect on the more central part of the vascular tree (*i.e.* on the part nearer the heart), changing to a dilator effect on more peripheral branches. The differences noted between its action in different species may then be regarded as due to differences in the level at which this change occurs. In the dog, and probably in the monkey, it occurs before the smallest macroscopic arteries are reached; in the cat it occurs more peripherally, so that the dilator effect is mainly, if not entirely, exercised on the capillaries; in the rodents it is pushed so far to the periphery as to escape detection, and possibly does not exist at all.

PART II. EFFECTS OBSERVED WITH NATURAL CIRCULATION.

We made a number of experiments on cats anæsthetised with ether, or decapitated under preliminary anæsthesia, in order to examine the possibility of restoring a failing vaso-dilator response to histamine, by injecting into the naturally circulating blood the vaso-constrictor substances which we had previously tested in this direction on the artificially perfused organ. The interpretation of the results proved to be so complicated that we do not propose to discuss them at present. The experiments led us, however, to the study of certain effects produced by histamine and by adrenaline, which seems to throw important light on the physiological antagonism between their respective types of action.

1. *Secondary pressor effect of histamine.* When the general arterial pressure of a spinal preparation has fallen to a low level, through failing efficiency of the spinal vaso-motor centres, injection of a small dose of histamine (such as 0·01 mgm.) still causes a definite, though often relatively trivial, further fall of the arterial pressure. This fall, however, is frequently followed by a secondary rise of the pressure, which may be extensive, and is habitually more conspicuous than the preliminary fall. Attention has been specially directed to this secondary phase of the action of histamine, under condition of low arterial pressure, by Hogben, Schlapp and Macdonald(18), who encountered it as a complication in determining the specific pressor action of certain pituitary extracts. We found that its relative prominence was exaggerated, if we restricted the vascular capacity of the spinal preparation by removing the stomach and intestine and excluding the liver from circulation. The phenomenon, as it was habitually observed in such preparations, is illustrated in Figs. 10 *A* and 11 *A*. In considering the nature of this pressor effect, we naturally had in mind the double action of histamine, as shown on the vessels of the perfused organ. Histamine has been shown to have a constrictor action on the small arteries of the cat, in addition to its more peripheral dilator action. Nothing in our experience with perfused organs, however, corresponded to this sequence of a dilator followed by a very powerful, delayed constrictor action. Inspection of the manometer, moreover, during the production of such records, revealed suggestive features, which are not clearly visible in the tracing on a slowly moving

surface. The initial fall of pressure was accompanied by such moderate acceleration of the heart-beat as normally accompanies the depressor

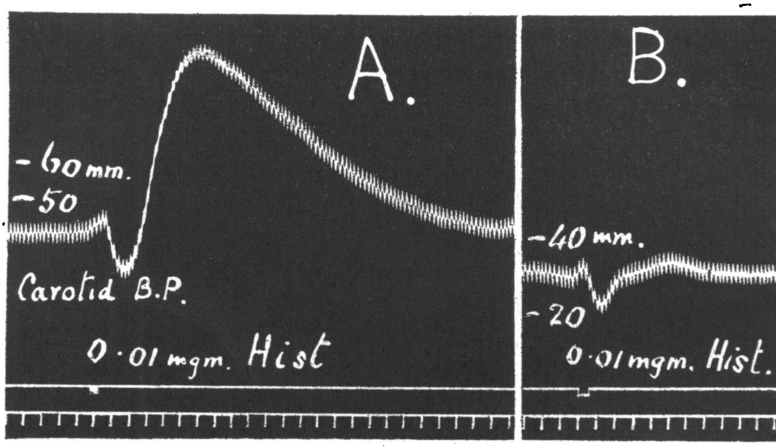

Fig. 10. Arterial pressure of spinal cat to show secondary pressor effect following injection of histamine; (*A*) before, (*B*) after extirpating suprarenal glands.

action of a small dose of histamine, and this passed off as the pressure curve turned upwards again. Soon after the turn, however, a second, much more pronounced acceleration of the heart began abruptly, and continued as the pressure rose rapidly to the secondary peak, subsiding during the return to the normal. This suggested the sudden entry into the circulation of an agent other than histamine. Inspection of plethysmograph records from a denervated limb reinforced this suggestion. During the primary fall of pressure, following injection of a small dose of histamine when the arterial pressure was low, the limb might show a small expansion or a small shrinkage of volume; concurrently with the secondary pressor phase the limb volume showed a secondary, much more pronounced and rapid shrinkage.

The fact, clearly demonstrated by Kellaway and Cowell(19), that a small intravenous injection of histamine is regularly followed by a brief acceleration of secretion from the suprarenal glands, suggested that the secondary pressor effect might be due to such a small gush of adrenaline, and not a direct effect of the histamine itself. The suggestion was supported by the observation that, when successive small injections of histamine were made into the vein of a spinal cat, at fairly short intervals, the secondary pressor effect became gradually smaller, but recovered when the preparation was left for a longer time without injection. The possibility was tested in two ways. In several experiments, in which the pressor effect was initially well marked (Fig. 10 *A*), the suprarenal glands were extirpated, with the result that subsequent injections of histamine produced small depressor effects, with practically no secondary pressor phase (Fig. 10 *B*). In another case, in which the pressor effect was unusually pronounced (Fig. 11 *A*), ergotamine was injected in

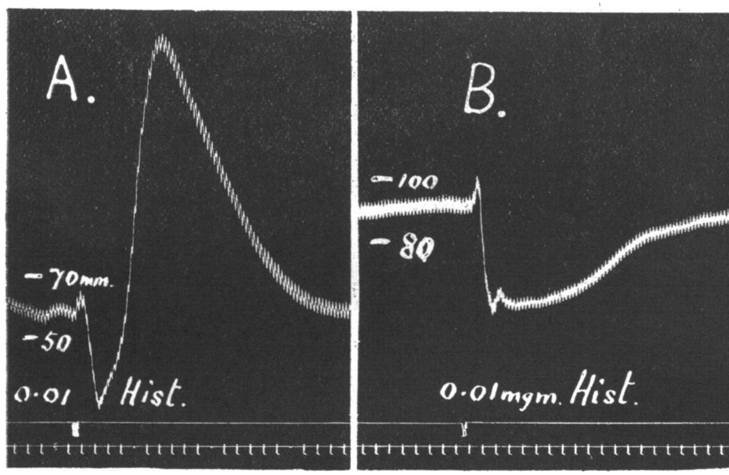

Fig. 11. Similar to Fig. 10; (*A*) before, (*B*) after ergotamine.

sufficient dose to reverse the pressor action of adrenaline, an injection of which now caused a simple fall of arterial pressure. Another dose of histamine was then injected, and in place of the pressor phase a secondary depressor action was produced, much more prolonged than that primarily caused by the histamine, and beginning just as the latter had passed its maximum (Fig. 11 *B*). This secondary depressor action was accompanied by acceleration of the heart-beat, such as adrenaline itself produces under the same conditions.

This evidence seems to put it beyond doubt that the delayed pressor action, which follows the injection of histamine under these conditions of low arterial pressure, is not a direct histamine effect at all, but is due to the suddenly accelerated output of adrenaline from the suprarenal glands. We believe the effect to be a really specific one. Though it has been shown that the adrenaline content of the suprarenal blood is somewhat increased by any influence which lowers the general arterial pressure, other depressor agents do not cause such an accelerated output as is represented by this pressor after-effect of histamine. An equipressor dose of acetyl-choline, for example, given under identical conditions, produces nothing comparable to it.

2. *The depressor effect of adrenaline.* The fact has long been known, that a very small dose of adrenaline, injected intravenously into an anæsthetised cat or dog with good vascular tone, will produce a predominantly depressor, vaso-dilator effect, the more familiar pressor effect of adrenaline forming, under such conditions, a trivial preliminary phase of the action. The nature of this effect was fully considered by Dale and Richards, who mentioned the earlier literature dealing with it. They were impressed by the similarity of this vaso-dilator action of adrenaline, in its distribution and in the conditions favouring its appearance, to that of histamine, and concluded that it was probably located on the same part of the peripheral vessels, *i.e.* on the capillaries in the cat. This conclusion produced a difficulty of conception which they were unable adequately to resolve. They were led by other considerations to the view, which our own evidence supports, that adrenaline is probably the most important factor, in the absence of nervous control, in maintaining in the cat the capillary tone which histamine inhibits. The vaso-dilator action of adrenaline being similar to that of histamine, we are then faced with the necessity of supposing that a steady content of adrenaline would maintain a tone, which a small, sudden, extra injection of the same substance would inhibit. We tested this not very easy supposition by giving a very slow, steady infusion of adrenaline into the vein of a cat, under conditions in which a small, sudden injection produced the vaso-dilator, depressor effect. The result is shown in Fig. 12. It was clear that adrenaline, artificially introduced at a slow steady rate into the venous

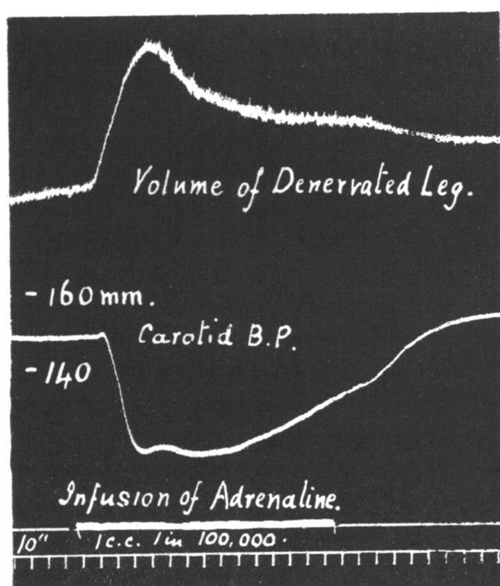

Fig. 12. Arterial pressure and volume of denervated leg of cat under ether. Very slow infusion of adrenaline into jugular vein.

blood, is able to produce a protracted, though limited fall in the peripheral resistance. The difficulty of attributing to adrenaline a steadily maintained tone, which a small, sudden dose will relax, appeared to be accentuated.

The evidence presented in the preceding section shows that the effect of histamine, injected into the circulation of the living animal, is complicated by a secondary action, not represented at all in its effects on the perfused organ, and due to the response of the suprarenal glands. Now the vaso-dilator effect of small doses of adrenaline, as seen in records of arterial pressure and denervated limb volume in the whole animal, is also a second phase of its action, following with a relatively long latent period a small, primary pressor effect, and it is similarly not represented by any demonstrable action of adrenaline on the perfused organ. If it could be shown that it was due to the output, in response to the adrenaline injection, of a small quantity of a substance having an action like that of histamine, the difficulties encountered in interpreting its meaning would disappear. The identity of the species showing vaso-dilator effects with histamine and with adrenaline, the correspondence of the conditions intensifying these two effects, and the failure to produce the vaso-dilator action of adrenaline on an isolated organ, would all be adequately explained.

If such an output of a histamine-like principle was concerned in this effect, there was no reason to expect that it would issue from a special glandular organ, as adrenaline does. On the contrary, evidence has accumulated during recent years, in specially convincing form in the work of Lewis and Grant(16), in favour of the view that such a principle is constantly being formed in all the tissues, its production being accelerated by slight injury of any kind, and that it plays an important part in regulating the capillary circulation. If its production, in response to injection of adrenaline, had this general distribution, there would be no obvious method of obtaining evidence of it. It occurred to us, however, that there is one organ, the lungs, through which adrenaline, given by the ordinary method of intravenous injection, has to pass, before it reaches the systemic circulation. In the lungs, moreover, adrenaline in these minute doses has never been shown to have any vaso-constrictor action. If any part of the depressor, vaso-dilator effect on the peripheral, systemic vessels should be due to a histamine-like substance shed into the blood from the lungs, in response to the passage of the adrenaline through their vessels, we should expect that the depressor effect would be larger, and that its latent period would be shorter, when the adrenaline is injected into a vein, than when it is injected, in equal dose, directly into the arterial stream. We have made the experiment six times, on cats under ether, and the result has uniformly confirmed this expectation.

Arterial pressure was in all cases recorded from a carotid artery. For the arterial injections we chose the left subclavian artery, since its separate origin, beyond that of the innominate, ensured that an injection made through its central stump into the aorta would all be carried caudally in the blood of the descending aorta. It was necessary only to tie off the branches arising from it between the cannula and its aortic origin, to prevent any part of the injection becoming side-tracked and failing to enter the aortic stream. We found it not difficult to tie off the thyroid axis, vertebral and internal mammary arteries, by dissection behind the pleura. A bull-dog clamp was then applied and a cannula tied centrally into the subclavian artery beyond it. A similar cannula was tied into the external or the internal jugular vein, just above its entrance into the superior vena cava. The cannulæ were filled with adrenaline solution of 1 in 100,000, and a small syringe containing the requisite small dose of the same solution was attached to one or the other. The clamp on artery or vein was then opened and the syringe rapidly emptied into the vessel. In one experiment we recorded also the volume of a hind leg, denervated a week previously by aseptic section of the sciatic and anterior crural nerves.

The results were in all cases the same. A small dose (0·002–0·004 mgm.) of adrenaline, injected into the vein, produced a small, sharp rise of arterial pressure, apparently due to acceleration of the heart, which was immediately succeeded by the characteristic depressor action. The same dose, injected into the aorta, usually caused a slighter vaso-constrictor rise of arterial pressure, which was sometimes so small as to be barely perceptible, but when visible was much more persistent than that caused by the intravenous injection. When this rise had subsided, there followed a depressor effect conspicuously smaller than that forming the main phase of the effect following intravenous injection, and sometimes so small as to be hardly detectable. A characteristic sequence of such effects is shown in Fig. 13 *A*, *B* and *C*.

Several possible explanations of this contrast had to be considered. We had to make sure, in the first place, that our arterial injections really delivered the full dose into the main arterial stream. This was controlled by injecting small doses of histamine through the same arterial and venous cannulæ. The depressor effects of these were indistinguishable

(Figs. 13 and 14 *D* and *E*), except by their latent periods, as presently to be mentioned. Then it might be suggested that the venous injection alone gave the adrenaline early access to the coronary vessels, as shown

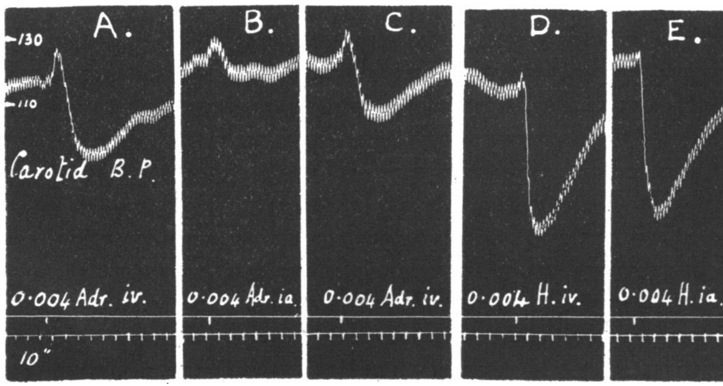

Fig. 13. Depressor effects of small doses of adrenaline in cat under ether, given intravenously (*A* and *C*) and intra-arterially (*B*). *D* and *E* show similar injections of histamine for comparison.

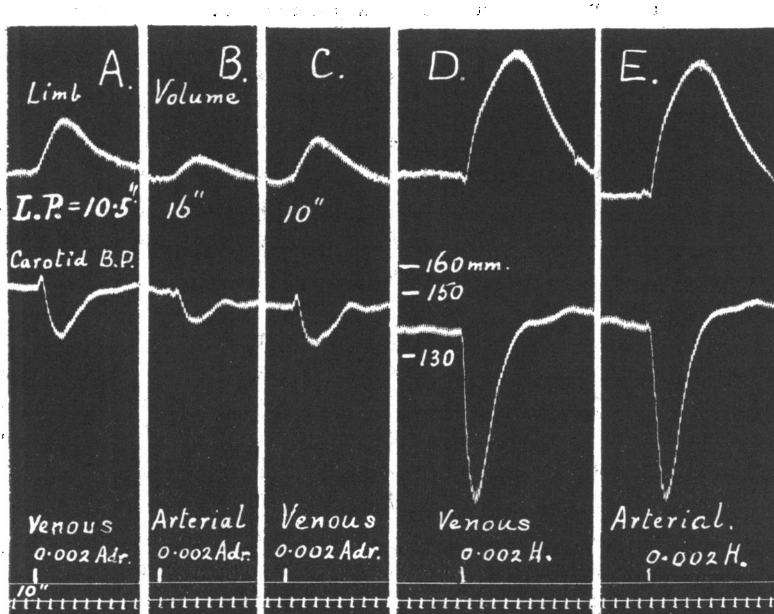

Fig. 14. Similar to Fig. 13, with plethysmograph record of volume of denervated limb.

by the accelerator effect on the heart, and that dilatation of the coronary vessels might cause the fall of carotid pressure; or, again, that the small dose of adrenaline, in passing through the lungs, produced sufficient additional resistance in their vessels to reduce the inflow, and therefore the output, on the left side of the heart. Both these explanations, on other grounds not very probable, appear to be completely excluded by the fact that the falls of arterial pressure are accompanied by corresponding dilatations of the denervated leg, as shown in Fig. 14 *A*, *B* and *C*, from which it can be seen that the volume-record displays an entirely similar contrast between the effects of arterial and venous injections of the same small dose of adrenaline.

The depressor effect following the arterial injection is not only smaller; the latent period of its onset is conspicuously longer. It is not easy to get exact measurements of the latent period of a depressor immediately following a pressor effect, owing to the difficulty of separating subsidence of the former from onset of the latter; but the difference is so conspicuous that great accuracy is not needed. In one case, in which measurements were made from a record on a rapidly travelling surface, the estimate for the latent period with venous injection was 9 seconds, with arterial injection 13 seconds; difference 4 seconds. On the volume record the small, preliminary pressor phase has usually no perceptible effect, and the beginning of the expansion can be detected with reasonable precision. One of us, making the injection, signalled to an assistant with a stop-watch as the piston was pressed home. The other, watching the volume record, signalled the onset of the dilatation. The following were the times recorded in seconds by this method.

Dose of adrenaline	Venous injection	Arterial injection	Difference
0·004 mgm.	7	14	7
		12	5
0·002 mgm.	11·5	17·5	6
	10	16	6
		Average	6 seconds

The differences are, in each case, between the latencies of consecutive injections. It will be seen that the latent periods with the smaller dose are uniformly longer than with the larger, but that the average differences between the arterial and venous delays are the same. The method of measurement, though crude, was sufficiently accurate, and the difference observed was widely outside its possible error.

We may safely conclude, therefore, that the latent period of the depressor effect following arterial injection is longer by some 4–6 seconds than that of the effect following the ordinary intravenous injection. When histamine, on the contrary, was injected by the same methods, we found, in confirmation of earlier observations (cf. Dale and Richards) that the depressor effect, and the increase of limb volume, began a second or two earlier with arterial than with venous injection, as would be expected of an effect due to direct, peripheral vaso-dilator action. The conclusion seems to be inevitable that the vaso-dilator effect produced by an injection of adrenaline is not due to an action of this type, but is secondary to the liberation, in response to the adrenaline injected, of a vaso-dilator substance. We should expect an effect of this secondary kind to have a relatively long latency in any case. On the supposition that the place of origin of the dilator substance is in the lungs, we should expect that a large part of an arterially injected dose of adrenaline would disappear before reaching them, and that the time taken to travel round the major circulation would involve an additional latency of some 4–6 seconds. The results observed confirm all these expectations.

3. *Discussion.* The results of these experiments strengthen the suggestion, which has frequently been made, that there is a special physiological antagonism between adrenaline, on the one hand, and a capillary-dilator principle, on the other hand, which closely resembles histamine in its action. Dale and Richards pointed out that the liberation of such a principle, as the result of metabolic activity, would provide a perfect fine adjustment of the capillary circulation to the needs of the tissues, and suggested that an important function of the normal output of adrenaline, from the suprarenal glands, might be so to balance and antagonise this dilator action as to maintain or restore a normal capillary tone. This conception seems to be supported by the evidence that each of these substances, injected in small dose, causes an accelerated output of its antagonist. Under appropriate conditions, a minute dose of histamine produces an effect in which the action of adrenaline is more prominent than that of the histamine itself, while a small dose of adrenaline, injected under the alternative conditions, produces an effect which is mainly of the histamine type, and apparently due to the output of a histamine-like principle. It is hardly likely that this natural output of each antagonist, in response to the sudden appearance in circulation of an excess of the other, occurs only under the conditions favouring its detection. It is, of course, obvious that low vascular tone will accentuate the secondary (adrenaline) effect of histamine in comparison with its direct depressor action, while high vascular tone will similarly accentuate the secondary (histamine) effect

following the injection of adrenaline, in relation to, and at the expense of, its direct vaso-constrictor action. We suppose, however, that under conditions rendering these secondary effects less obvious, the effect of a dose of either of these substances is really modified, and rendered more evanescent, by a compensatory output of its antagonist. The direct effect of either substance on the arterial pressure, even under conditions not specially favouring the effect of its antagonist, can, indeed, be observed, in many cases, to be followed by a swing of the pressure in the opposite direction, before equilibrium at the original level is re-established. That such a compensation is normally in action is, further, strongly suggested by the observation, already mentioned, of the greatly accentuated effect of a small dose of histamine when the adrenals, or only their medullary portions, have been extirpated.

We have presented evidence which seems to point to the lungs, in particular, as a source of a histamine-like antagonist to adrenaline. It should be made clear, however, that our evidence only deals with the response to a sudden artificial injection of adrenaline, by output of a depressor substance in such quantity as to over-compensate the direct adrenaline effect. The facts do not warrant the suggestion that the lungs are the only, or even the principal place of origin of such a substance under physiological conditions. As to the part they might play in relation to the passage through them, on its way to the major circulation, of adrenaline issuing at normal rate from the suprarenal gland, we have no material even for conjecture.

There are many points in this connection which can only be elucidated by further experiment. We must content ourselves, for the present, with the presentation of evidence which reinforces the suggestion that action of the histamine type is no mere pharmacological curiosity, but one of genuine physiological importance, in providing, at least in many species, one side of the balanced chemical control of capillary tone, of which the other is provided chiefly by the natural secretion of adrenaline.

SUMMARY.

1. The vessels of a cat's limb, artificially perfused with blood, are relaxed by histamine. The tone favouring this reaction is evanescent, but can regularly be restored by a trace of adrenaline, and occasionally by pituitary extract.

2. The vessels of the limb of a monkey or dog, artificially perfused, are relaxed by histamine, and the reaction can be obtained repeatedly.

3. The perfused arterioles of the cat are constricted, those of the dog

relaxed by histamine. It is suggested that the predominant incidence of the histamine dilator action on the capillaries is peculiar to the cat.

4. There is evidence that a secondary pressor effect of histamine is due to accelerated output of adrenaline; and that the depressor effect of small doses of adrenaline, seen in the anæsthetised cat or dog, is also a secondary effect, due to liberation of a histamine-like principle.

5. The bearing of these observations on the chemical control of capillary tone is discussed.

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